the first stage, except that unsensitized guinea pig controls are not necessary. The results are evaluated by combining the results obtained on all guinea pigs tested in stages one and two. Calculate the average response on the 20 *M. bovis* sensitized animals and on the 20 *M. avium* sensitized animals and determine the specificity index. An inconclusive serial is satisfactory after the second stage test, if its specificity index is 400 square millimeters or more, and unsatisfactory if its specificity index is less than 400 square millimeters.

(d) *Special chemical tests and requirements.* Final container samples of completed product from each serial shall be tested as follows:

(1) *Protein concentration.* The final product shall contain a protein concentration of 1.0 ±0.1 mg/ml. The Microkjeldahl Test for Nitrogen shall be used.

(2) *Phenol content.* Phenol content of the final product shall be 0.50 percent plus or minus 0.04 percent. A direct titration with a standardized bromide-bromate solution shall be conducted.

[41 FR 8471, Feb. 27, 1976, as amended at 41 FR 21760, May 28, 1976; 41 FR 32883, Aug. 6, 1976. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991]

ANTIBODY PRODUCTS

§ 113.450 General requirements for antibody products.

Unless otherwise prescribed in a Standard Requirement or in a filed Outline of Production, all antibody products shall meet the applicable requirements of this section.

(a) *Terminology.* The following terms in the regulations and standards concerning antibody products shall mean:

Antibody. An immunoglobulin molecule, having a precise glycoprotein structure, produced by certain cells of the B lymphocyte lineage in response to antigenic stimulation, and functioning to specifically bind and influence the antigens that induced its synthesis.

IgG (Immunoglobulin G). One of the several recognized classes of structurally related glycoproteins whose representatives include all known antibodies.

Monoclonal. Produced by, or derived from, the offspring of a single common progenitor cell.

Failure of passive transfer. A condition of neonates characterized by an abnormally low concentration of circulating maternal IgG.

(b) *Nomenclature.* Antibody products shall be named as follows:

(1) *Virus-specific products.* The true name of a virus-specific product shall: include the term ''antibody,'' specify the disease for which the product is intended, and indicate the type of animal that supplied the component antibodies. If the antibodies are monoclonal, the term ''monoclonal'' shall be used. Example: ''Duck Virus Hepatitis Antibody, Duck Origin.''

(2) *Bacterium-specific products.* The true name of a bacterium-specific product shall: include the term ''antibody'' if the component antibodies are directed against a nontoxin antigen or the term ''antitoxin'' if the component antibodies are directed against toxin, specify the organism against which the product is intended, and indicate the type of animal that supplied the component antibodies. If the antibodies are monoclonal, the term ''monoclonal'' shall be used. Example: ''Escherichia Coli Monoclonal Antibody, Murine Origin.''

(3) *Failure of passive transfer products.* The true name of a product for treatment of failure of passive transfer shall include the term ''IgG'' and indicate the type of animal that supplied the component IgG. Example: ''Bovine IgG.''

(4) *Combination products.* The true name of a product for treatment of failure of passive transfer as well as for the prevention and/or alleviation of a specific viral or bacterial disease shall be named according to the nomenclature prescribed above for virus-specific or bacterium-specific products.

(c) *Animals.* All animals used in the production of antibody products shall be healthy. Their health status shall be determined by physical examination by, or under the direct supervision of, a licensed veterinarian and by tests for infectious diseases. Such animals shall be maintained at licensed establishments: *Provided,* That cows maintained at Grade A dairies (or the equivalent)

that are not injected with antigens for the purpose of stimulating the production of specific antibodies and that are used only for the purpose of supplying lacteal secretions are exempt from being maintained at a licensed establishment.

(1) No animal shall be used while showing clinical signs of disease. The presence of minor localized injuries or lesions (contusions, lacerations, burns, etc.) without body temperature elevation and without significant pain and distress shall not be construed as clinical evidence of disease.

(2) Before first use and on a regular basis, all animals used in the manufacture of antibody products shall be individually subjected to applicable tests for infectious diseases. Records of all test results shall be maintained. An animal which tests positive for an infectious disease shall not be used in the manufacture of antibody products. Retests shall be conducted as deemed necessary by the Administrator.

(i) Before first use, horses shall be tested as follows for:

(A) Equine infectious anemia (EIA) at a laboratory approved by APHIS.

(B) Piroplasmosis, dourine, and glanders at the National Veterinary Services Laboratories.

(C) Brucellosis at a laboratory approved by APHIS. Horses with standard agglutination titers of 1:50 or less can be used for production. Horses with standard agglutination titers equal to or greater than 1:100 may be tested by the Rivanol or card tests. Reactors to these supplemental tests shall not be used for production. Nonreactors to the supplemental tests shall be retested after 30 days. If the supplemental tests are negative and the agglutination titer has not increased, the animal may be used for production. Otherwise, the animal is unsatisfactory for this purpose.

(ii) Horses shall be retested annually for EIA and, if housed or pastured with any other species, shall be retested annually for brucellosis.

(iii) Before first use, cattle shall be tested as follows for:

(A) Tuberculosis by an accredited veterinarian: *Provided,* That cattle at Grade A dairies supplying only lacteal secretions need only be tested for tuberculosis in accordance with applicable Milk Ordinances or similar laws or regulations.

(B) Brucellosis at a laboratory approved by APHIS. Cattle with standard agglutination titers of 1:50 or less can be used for production. Cattle with standard agglutination titers equal to or greater than 1:100 may be tested by the Rivanol or card tests. Reactors to these supplemental tests shall not be used for production. Nonreactors to the supplemental tests shall be retested after 30 days. If the supplemental tests are negative and the agglutination titer has not increased, the animal may be used for production; otherwise, the animal is unsatisfactory for this purpose. Cattle at Grade A dairies supplying only lacteal secretions need not be tested individually for brucellosis if a portion of their secretions contribute to the herd milk pool tested as required by the brucellosis ring test. An animal of a herd testing positive by this test shall not be used in production.

(iv) Cattle shall be retested annually for both tuberculosis and brucellosis. Cattle at Grade A dairies supplying only lacteal secretions need only be tested for tuberculosis in accordance with applicable Milk Ordinances or similar laws or regulations. Cattle at Grade A dairies supplying only lacteal secretions need not be tested individually for brucellosis if a portion of their secretions contribute to the herd milk pool tested as required by the brucellosis ring test. An animal of a herd testing positive by this test shall not be used in production.

(v) For other species, appropriate tests and the frequency with which they are applied shall be specified in the filed Outline of Production for the product.

(vi) If a positive result is obtained on any prescribed test, the positive animal(s) shall be removed from the herd and the remaining animals retested. Production shall not be renewed until a negative herd test is obtained not less than 28 days following removal of the positive animal(s).

(vii) Negative animals shall be maintained separate and apart from untested or positive animals of any species. Production animals shall not be used

for any other purpose, such as testing, work, or recreation.

(d) *Collection procedures.* Blood, lacteal secretions, and egg material shall be collected as described in the filed Outline of Production for the product.

(e) *Ingredient handling and processing.* Blood derivatives (serum, plasma, etc.), lacteal secretions, and egg material used in the production of antibody products shall be subjected to an appropriate procedure for the inactivation of potential contaminating microorganisms. The procedure shall be one of those described below and specified in the filed Outline of Production for the product: *Provided,* That another procedure may be substituted if demonstrated to be at least as effective by data acceptable to APHIS and specified in the filed Outline of Production for the product. These data are expected to come from a study comparing the effectiveness of the established and substitute procedures against a satisfactory battery of potential contaminating microorganisms.

(1) Blood derivatives of equine origin shall be heated at 58.0–59.0°C for 60 minutes, and blood derivatives of bovine, porcine, or other origin shall be heated at 58.0–59.0°C for 30 minutes. In lieu of heat treatment, blood derivatives of any origin may be treated with at least 2.5 megarads of ionizing radiation, with a maximum radiation dosage specified in the filed Outline of Production for the product.

(2) Lacteal secretions shall be heated as described in paragraph (e)(1) of this section, or shall be pasteurized at either 72°C for 15 seconds or 89°C for 1 second using appropriate equipment. In lieu of the heat treatment regimens prescribed, lacteal secretions may be treated with at least 2.5 megarads of ionizing radiation, with a maximum radiation dosage specified in the Outline of Production for the product.

(3) Egg material shall be heated at 58.0–59.0°C for 30 minutes, or treated with at least 2.5 megarads of ionizing radiation, with a maximum radiation dosage specified in the filed Outline of Production for the product.

(4) Blood derivatives, lacteal secretions, and egg material shall not contain preservatives at the time of heat treatment, and immediately after heat treatment shall be cooled to 7°C or lower.

(5) Licensees shall keep detailed records as to each batch treated and each serial of product prepared for marketing. Recording charts shall bear full information concerning the material treated and tests made of the equipment used for treatment.

(f) *Preservatives.* Liquid antibody products, except those immediately frozen following preparation and maintained in a frozen state until time of use, shall contain at least one preservative from the following list, within the range of concentration set forth:

(1) Phenol 0.25 to 0.55 percent, or

(2) Cresol 0.10 to 0.30 percent, and/or

(3) Thimerosal 0.01 to 0.03 percent, or

(4) Other preservative(s) specified in the filed Outline of Production for the product.

(g) *Antigens for hyperimmunization.* If animals are hyperimmunized to generate antibodies for a product for the prevention and/or alleviation of a specific infectious disease, and a USDA-licensed veterinary biological product is not employed for this purpose, the following shall apply:

(1) For each antigen, a Master Seed shall be established.

(i) Bacterial Master Seeds shall be tested for purity and identity as prescribed for live bacterial vaccines in § 113.64.

(ii) Viral Master Seeds shall be tested for purity and identity as prescribed for live virus vaccines in § 113.300.

(2) The maximum allowable passage level of the hyperimmunizing antigen shall be the passage level of the antigen used to generate product shown to be efficacious and shall not exceed 10 passages from the Master Seed.

(h) *Purity tests.* Final container samples of each serial and each subserial shall be tested for viable bacteria and fungi as follows:

(1) Dried products for parenteral administration and liquid products shall be tested as prescribed in § 113.26.

(2) For dried products for oral administration, 10 final container samples shall be reconstituted with sterile water at the volume recommended on the label and tested for the following contaminants:

(i) *Coliforms.* One milliliter of each rehydrated sample shall be pipetted into a 100×15 mm petri dish and 10–15 ml of violet red bile agar at 45–50°C added. The plate shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 35°C for 24 hours. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If characteristic growth is observed on the negative control plate, or no characteristic growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If characteristic growth is observed on any of the 10 plates containing samples of the serial, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If characteristic growth is observed on any of the retest plates, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(ii) *Salmonellae.* One milliliter of each rehydrated sample shall be pipetted into a 100×15 mm petri dish and 10–15 ml of brilliant green agar at 45–50°C added. The dish shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 35°C for 24 hours. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If characteristic growth is observed on the negative control plate, or no characteristic growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If characteristic growth is observed on any of the 10 plates containing samples of the serial, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If characteristic growth is observed on any of the retest plates, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(iii) *Fungi.* One milliliter of each rehydrated sample shall be pipetted into a 100×15 mm petri dish and 10–15 ml of appropriately acidified potato dextrose agar at 45–50°C added. The plate shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 20–25°C for 5 days. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If growth is observed on the negative control plate, or no growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If growth is observed on any of the 10 plates containing samples of the serial, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If growth is observed on any of the retest plates, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(iv) *Total bacterial count.* One milliliter of each rehydrated sample, undiluted or diluted as prescribed in the Outline of Production, shall be pipetted into a 100×15 mm petri dish and 10–15 ml of tryptone glucose extract agar at 45–50°C added. The plate shall be manipulated to coat its entirety with the agar-sample mixture and allowed to stand until the mixture solidifies. The plate shall then be incubated at 35°C for 48 hours. A positive control plate and a negative control plate shall be prepared at the same time and in the same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If growth is observed on the negative control plate, or no growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If the average number of bacterial colonies on the 10 plates containing samples of the serial exceeds that specified in the filed Outline of Production for the product, one retest to rule out

faulty technique may be conducted on samples from 20 final containers. If the average number of bacterial colonies on the retest plates exceeds that specified in the filed Outline of Production for the product, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(i) *Safety tests.* Bulk or final container samples of each serial shall be tested as prescribed in § 113.33(b). Dried product shall be reconstituted as indicated on the label and 0.5 ml injected per mouse.

[61 FR 51774, Oct. 4, 1996]

§ 113.451 Tetanus Antitoxin.

Tetanus Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium tetani.* Each serial shall meet the applicable general requirements provided in § 113.450 and paragraph (a) of this section, and be tested for potency as provided in paragraph (b) of this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *General requirements.* The amount of antitoxin in a final container shall be the amount which is delivered from such container when opened and inverted until the flow stops. A graduated volumetric cylinder which conforms to the National Institute of Standards and Technology requirements shall be used. The reading shall be made at the bottom of the meniscus. Volumes of 10 ml or less shall be recorded to the nearest 0.1 and volumes over 10 ml shall be recorded to the nearest ml.

(1) All final containers of Tetanus Antitoxin shall yield not less than the labeled unitage of antitoxin throughout the dating period. The minimum package size permitted for marketing in the United States shall be a 1,500 unit vial.

(2) The expiration date of Tetanus Antitoxin shall be not more than 3 years after the date of a potency test which demonstrates that the recoverable antitoxin from the final container provides at least 20 percent excess over the number of units claimed on the label or not more than 1 year after the date of a potency test which demonstrates that the recoverable antitoxin from the final container provides 10 to 19 percent excess over the number of units claimed on the label.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be assayed to calculate the units of Tetanus Antitoxin in each final container. A comparative toxin-antitoxin neutralization test shall be conducted using a standard antitoxin and a standard toxin. All dilutions shall be made in M/15 phosphate buffered (pH) 7.4 physiological saline with 0.2 percent gelatin.

(1) One ml of the Standard Antitoxin shall be diluted before use so the final volume contains 0.1 unit per ml. The dilution shall be held at 20° to 25 ° C for 30 minutes prior to combination with a test does of toxin.

(2) The Standard Toxin test dose is that amount which when mixed with 0.1 unit of Standard Antitoxin, incubated at 20° to 25 ° C for 1 hour, and injected subcutaneously into a 340 to 380 gram guinea pig, results in death of that guinea pig within 60 to 120 hours with clinical signs of tetanus. The toxin shall be diluted so the test dose shall be in 2.0 ml.

(3) A mixture of diluted Standard Toxin and diluted Standard Antitoxin shall be made so that 0.1 unit of antitoxin in 1 ml is combined with a test dose of toxin. This Standard Toxin-Antitoxin mixture shall be held at 20° to 25 ° C for 1 hour before injections of guinea pigs are made.

(4) A sample from each serial of antitoxin shall be prepared as was the Standard Toxin-Antitoxin mixture; except the amount of antitoxin shall be based on an estimation of the expected potency. When testing is done on bulk material, the final container fill shall reflect the endpoint value plus 10 percent overage for 1 year dating and 20 percent overage for 3 year dating.

(5) Normal guinea pigs weighing within a range of 340 to 380 grams shall be used. Pregnant guinea pigs must not be used.

(i) Each of two guinea pigs (controls) shall be injected subcutaneously with a 3 ml dose of the Standard Toxin-Antitoxin mixture. Injections shall be made in the same order that toxin is added to the dilutions of antitoxins. These